Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	"5458518".pn.	US-PGPUB; USPAT	OR	OFF	2005/01/21 14:49
L2	12	("5228878" "5389026").PN. OR ("5458518").URPN.	US-PGPUB; USPAT; USOCR	OR	OFF	2005/01/21 14:52
L3	42	("3665241" "3755704"	US-PGPUB;	OR	OFF	2005/01/21 14:59
		"3812559" "3954523" "4016017"	USPAT;		45-4	
		"4266233" "4652467" "4857161" "4987101" "5103288"	USOCR			
		"5142184" "5186670" "5194780" "5229331" "5259799"				
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		"5473222" "5483067" "5529524" "5569058" "5578896" "5585301" "5597444"				
		"5653619" "5663608" "5684356" "5712534" "5793154"				
		"5804910" "5853492" "5869169" "5898258" "6028322"				
		"6232705" "6251470" "6255156" "6277765" "6333215").PN. OR				
		("6835111").URPN.				

patent v vis 795 under ATCC No. CRL 8001 with the American log university Bouldard Wangs cs, VA 2010-2209

Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852, on April 26, 1979. OKT3 has been used for a long , time to suppress a T-cell response thus preventing rejection transplahts (Thistlethwaite al., Transplantation 38, 695-701 pp. (1984);Woodle Transplantation 51, pp. 1207-1212 (1991)). On the other can also thigger T-cell activation proliferation, which stimulates the effector cells, can be used for the adoptive\cancer immunotherapy (Yannelly et al., J. Immunol. Meth. $\underline{1}$, pp. 91-100 (1990)). OKT3 was used as such and as a component of a bispecific antibody to direct cytotoxic T-lymphocytes against tumor cells or virusinfected cells (Nitta et al. Lancet 335, pp. 368-376 (1990); Sanna et al., Bio/Technology 13, 1221-1224 pp. (1995)).Furthermore, humanized versions of the OKT3monoclonal antibody which were expressed in COS cells are also known (Woodle et al., J. Immunol. 148, pp. 2756-2763 (1992); Adair et al., Human. Antibdd. Hybridomas, pp. 41-47 (1994)). So far there has been the problem that OKT3 has no



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INS andia sufficient stability and particularly cannot be expressed in known recombinant expression systems in stably fashion and sufficient amount

Therefore, the object of the present invention was to express OKT3 recombinantly and obtain an antibody which has satisfactory stability.

This object is achieved by the subject matters defined in the claims.

The inventors have found that by introducing a point mutation at position H100A of the amino acid sequence of OKT3 the stability increases many times over. This point mutation relates to the exchange of cysteine for another polar amino acid, preferably serine, in the amino acid sequence of OKT3.

For the production of an antibody according invention, mRNA from freshly subcloned hybridoma cells of OKT3 is used as a basis. The cDNA is produced according to methods\known to a person skilled in the art, which were described in Dübel et al., J. Immunol. Methods 175, pp. 89-95 (1994), for example. The DNA coding for the variable domain of the light chain can be produced by means of PCR using suitable primers, e.g. by means of primers Bi5 and Bi8 which hybridize to the amino-terminal part of the constant domain of the k-chain and the framework 1 (FR1) region of the variable domain of the κ -chain (Dübel et al., above). For the amplification of the DNA which codes for the variable domain of the heavy chain, it is possible to use e.g. the primer Bi4 which hypridizes to the amino-terminal part of the constant domain 1 δf the γ -chain (Dübel et al., cf. above) and the primer Bi3f which hybridizes to the FR1 region of the heavy chain (Gotter e^{λ} al., Tumor Targeting $\underline{1}$, pp. 107-114 (1995).

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Thereafter, the amplified DNA is inserted in a vector adapted for sequencing and for site specific mutagenesis, as well known to the person skilled in the art. For example, vector pCR-Skript SK(+) sold by the Stratagene can be used. Mutations are inserted in the V_{μ} domain originating from OKT3 by site specific mutagenesis. The person skilled in the art is familiar necessary for this purpose, conditions they are also described e.g. in Kunkel et al., Meth. Enzymol. 154, pp. 367-382 (1987). The amino acid substitution at the H100A position of OKT3 (exchange of cysteine) is suitably carried out by using the primer SK1 5'-GTAGTCAAGGCTGTAATGATCATC if exchange for serine shall be carried out at this position.

Then, the thus modified DNA can be cloned into a vector and expression vector, respectively. The person skilled in the art is familiar with examples thereof. In the case of an expression vector, these are pGEMEX, pUC derivatives or pET3b. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, cDM8 and pCEV4 have to be indicated for the expression in animal cells. The baculovirus expression vector pAcSGHisNT-1 is especially suitable for the expression in insect cells. The expression in E. coli is preferred according to the invention, for which purpose preferably the vector pHOG21 shown in figure 1 (Kipriyanov et al., J. Immunol. Methods 196, pp. (1996) is used, in which the mutated OKT3 single chain gene is inserted as NcoI/BamHI DNA fragment. single-chain antibody OKT3 mutated at position 100 A (Kabat numbering system) is expressed, which has the sequence shown in figure 2.

The person skilled in the art is familiar with cells adapted to express a DNA which is present in an expression vector. Examples of such cells comprise the *E. coli* strains HB101, DH1, x1776, JM101, JM109, BI21 and SG13009, the yeast strain Saccharomyces cerevisiae and the animal cells 3T3, FM3A,

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CHO, COS, Vero and HeLa as well as the insect cells sf9. The use of the XL1-Blue $E.\ coli$ cells sold by the company of Stratagene is preferred.

The person skilled in the art knows in which way a DNA has to be inserted in an expression vector. He is also familiar with the fact that this DNA can be inserted in combination with DNA coding for another protein and respectively, so that the DNA can be expressed in the form of a fusion protein, e.g. in the form of a His fusion The information necessary for protein. this purpose included in the preferably used plasmid pHOG21. Furthermore, the mutated form of OKT3 can be present in the form of a bispecific antibody, e.g. in combination with an antibody human CD19 complex. The sequence of bispecific antibody is shown in figure 3.

Antibodies according to the invention distinguish themselves in that they can be produced by means of recombinant methods sufficient amount and have a stability greater compared to the non-mutated monoclonal antibody OKT3. This stability expresses itself e.g. in that the mutated antibody has lost almost nothing of its original binding affinity even after one month of storage at 4°C in PBS, whereas OKT3 has markedly lost binding affinity under these conditions (46 %). In addition, the antibody according to the invention has the advantage that as a single-chain antibody (scFv) it faster blood clearance and better tumor penetration. Furthermore, ScFvs are very useful molecules to transport pharmacons, toxins or radionuclides to tumor sites, which is important for tumor diagnosis and tumor treatment.

The present invention is further described by means of the figures.

Figure 1:

plasmid pHOG21

the abbreviations used therein having the following meanings:

Ap^R: ampicillin resistance gene